Remarks

Applicants have received and reviewed the Office Action dated June 15, 2000. By way of response, Applicants have cancelled claims 2, 13 and 14 without prejudice, amended claims 1, 3 - 5, 7, 9 and 17, and added new claims 18 - 23. Claims 1, 3 - 12, and 15 - 23 are pending. No new matter is introduced. Applicants submit that the amended and newly presented claims are supported by the specification.

In particular, support for the recitation in the claims regarding residues of aspargine-38 can be found in the specification at least at page 10, lines 9 - 12. A mutation can involve a change to one of the other 19 amino acids or a non-naturally occurring amino acid. Additional support is on page 11, lines 12 - 22 describing modifications to β-barrel 4 of B-subunit 5. The amendment to claim 1 reciting an amino acid substitution in a β-barrel of a B-subunit or a N-terminal alpha helix can be found in the specification at least at page 11, line 12 to page 13, line 24.

For the reasons given below, Applicants respectfully submit the amended and newly presented claims are in condition for allowance, and notification to that effect is earnestly solicited.

Petition for Extension of Time

A two-month extension of time is necessary to provide the response. A request is made for an extension of time from September 15, 2000 to November 15, 2000 for responding to the office action.

Claim Objections

Claim 17 was amended to remove the word "specie."

35 USC § 103 Rejections

The Examiner rejected claims 1 - 10, 13 - 14 and 17 under 35 USC § 103(a) as obvious over *Goshorn et. al.* (Infection and Immunity: 56(9): 2518-2520 (1988)) in view of *Hartwig et. al.* (International Immunology, 5(8): 869-875 (1993)). Applicants respectfully traverse this rejection.

Goshorn et al. does not disclose, nor does it discuss, the secondary structure of the mutant SPE-C toxin recited in claim 1. Rather, *Goshorn et al.* teaches only the primary structure of the SPE-C toxin by disclosing the amino acid sequence for SPE-C. There is no teaching of any particular mutation that would be substantially nonlethal compared with a protein substantially corresponding to wild type SPE-C toxin.

Hartwig et al. does not remove the deficiencies of *Goshorn et al.* *Hartwig et al.* discloses several mutations of SPE-A and the effect of these mutations on T lymphocyte stimulatory activity. Replacements of several amino acids of SPE-A with alanine were carried out by site-specific mutageneis. There are some similarities between the structures of SPE-A and SPE-C but they are different proteins and *Hartwig* discloses nothing about SPE-C. None of the mutations reported by *Hartwig et al.* for SPE-A correspond to the residues recited in the present claims. The residues disclosed by *Hartwig* are about 40 or more amino acids away and on a different part of a different protein. *Hartwig et al.* only discloses mutations to SPE-A and does not suggest any mutations to a different protein such as SPE-C, particularly to a portion of the protein that does not correspond to where the mutations were made in SPE-A. There is no suggestion that non-lethal mutations of SPE-C can be prepared by substituting an amino acid in a ß-barrel of a B-subunit or a N-terminal alpha helix. There is suggestion that one type of secondary structure is preferable for making nonlethal mutations of SPE-C. *Hartwig et al.* does not suggest specific mutations corresponding to aspartic acid-12, tyrosine-15, tyrosine-17, histidine-35, or asparigine-38 in SPE-C. Rather, *Hartwig* suggests mutations at a different part of a different protein.

The combination of references does not disclose or suggest the important characteristic of non-lethality. Rather, the combination of *Goshorn et al.* and *Hartwig et al.* only suggests that mutations can be made and that some mutations might affect T lymphocyte activity. However, disclosing how to produce a mutation does not teach how to make a mutation that is non-lethal. The combination of references does not suggest that altering amino acids in a ß-barrel of a B-subunit or a N-terminal alpha helix can produce non-lethal mutations of SPE-C. Further, the combination of references does not suggest the specific residues claimed in the present invention.

Accordingly, it is respectfully submitted that the combination of *Goshorn et al.* and *Hartwig et al.* does not make the present invention obvious. Withdrawal of this rejection is respectfully requested.

The Examiner also rejected claims 11 - 12 and 15 - 16 under 35 USC § 103(a) as obvious over *Goshorn et. al.* in view of *Hartwig et. al.* and *Leung et al.* (U.S. Patent No. 5,460,813). Applicants respectfully traverse this rejection.

Claims 11 - 12 and 15 - 16 are dependent on claim 1. *Leung et al.* does not remove the deficiencies noted above for the combination of *Goshorn et al.* with *Hartwig et al.* with respect to claim 1. That is, *Leung et al.* does not teach or suggest that altering amino acids in a ß-barrel of a B-subunit or a N-terminal alpha helix can produce a non-lethal SPE-C toxin. *Leung et al.* teaches modified TSST-1 as a vaccine against the action of TSST-1. However, this reference does not teach any particular mutations, of TSST-1. Further, this reference does not disclose or suggest the particular residues and structural features of SPE-C recited in the amended and newly presented claims.

Accordingly, it is respectfully submitted that the combination of *Goshorn et al.*, *Hartwig et al.* and *Leung et al.* does not make the present invention obvious. Withdrawal of this rejection is respectfully requested.

35 USC § 112, First Paragraph Rejections

The Examiner rejected claims 1 - 17 based on 35 USC § 112, first paragraph alleging insufficient support for every possible insertion, deletion or substitution of one or more amino acids. Applicants respectfully traverse this rejection.

Applicants note that the amended and newly presented claims relate to mutants of SPE-C including particular substitutions at particular amino acids or in particular structural domains. Each of these residues and structural domains are specifically called out in the present specification as preferred locations for substitutions. For example, the specification supports the recitation in claim 1 of a mutant comprising an amino acid substitution in a β-barrel of a B-subunit or a N-terminal alpha helix. At least page 11, lines 12 -22 of the specification supports mutations on β-Barrel 4 of B-subunit 5. Particular amino acids that are supported as mutation points are His-35, Asn-38, Thr-33 and Leu-36. At least page 13, lines 16 - 24 of the specification supports mutations on N-terminal alpha helix 51. Particular amino acids that are supported as mutation points are Ser-11, Asp-12, Tyr-15 and Tyr-17. Further, Example 6 (pages 36 - 40) provides support for a number of single and double mutants of SPE-C: Y17A, N38A, N38A, Y15A/N38A and Y17A/N38A. This description of suitable regions of the protein is

disclosure commensurate with the scope of the claim for producing nonlethal mutations. The claims recite mutations in these secondary structural features.

The Examiner alleges that the specification does not provide guidance on how multiple amino acids can be deleted, substituted or inserted for the production of a stable protein. The Applicants respectfully disagree regarding the relevancy of protein stability for this invention. As pointed out in the response to the first office action, the critical issue for a mutant to function as a vaccine is nonlethality rather than stability. The protein does not have to remain intact to function as a vaccine. There is support for claims that the mutants can be used as vaccines. Two double mutants (Y15A/N38A and Y17A/N38A) were prepared as described in Example 5 and then evaluated in Example 6. The mutations were effective as vaccines. Finally, there are no claims in the present invention regarding the stability of the mutations.

The Examiner further alleges that there is no substantive evidence that the claimed vaccines are capable of inducing protective immunity. Applicants respectfully disagree. The double mutants Y15A/N38A and Y17A/N38A were prepared in Example 5 and tested for capacity to enhance endotoxin shock in Example 6. After giving a group of rabbits either a mutant SPE-C toxin or SPE-C wild type toxin, the animals were challenged with *Salmonella typhimurium* endotoxin. The rabbits that had been given mutant SPE-C toxin survived whereas those that had received the wild-type toxin died (see Table 4 on page 37).

Further studies involved immunization of rabbit groups with 2 weekly doses of SPE-C double mutants. Blood samples before and after immunization were compared for antibodies against streptococcal derived wild type SPE-A. The blood samples after immunization had higher levels of antibodies (see Table 5 on page 38).

The immunized animals were then challenged with wild type SPE-C and then 4 hours later with *Salmonella typhimurium* endotoxin. None of the rabbits that were immunized died whereas rabbits that were not immunized died (see Table 6 on page 39).

By describing the discrete residues and specific structural features of the protein that are suitable for making mutations that yield a nonlethal SPE-C as well as showing specific examples of mutations that caused immunization against wild type SPE C and endotoxin, Applicants have met the standard for enablement under § 112, first paragraph.

Applicants respectfully submit that the claims are fully enabled by the specification and request withdrawal of this rejection.

Summary

Each of claims 1, 3 - 12, 15 - 22 are in condition for allowance. The Examiner is invited to contact Applicants' undersigned representative at the telephone number listed below if doing so will expedite the prosecution of this patent application.

Respectfully submitted,

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Date: Nov 15, 2000

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